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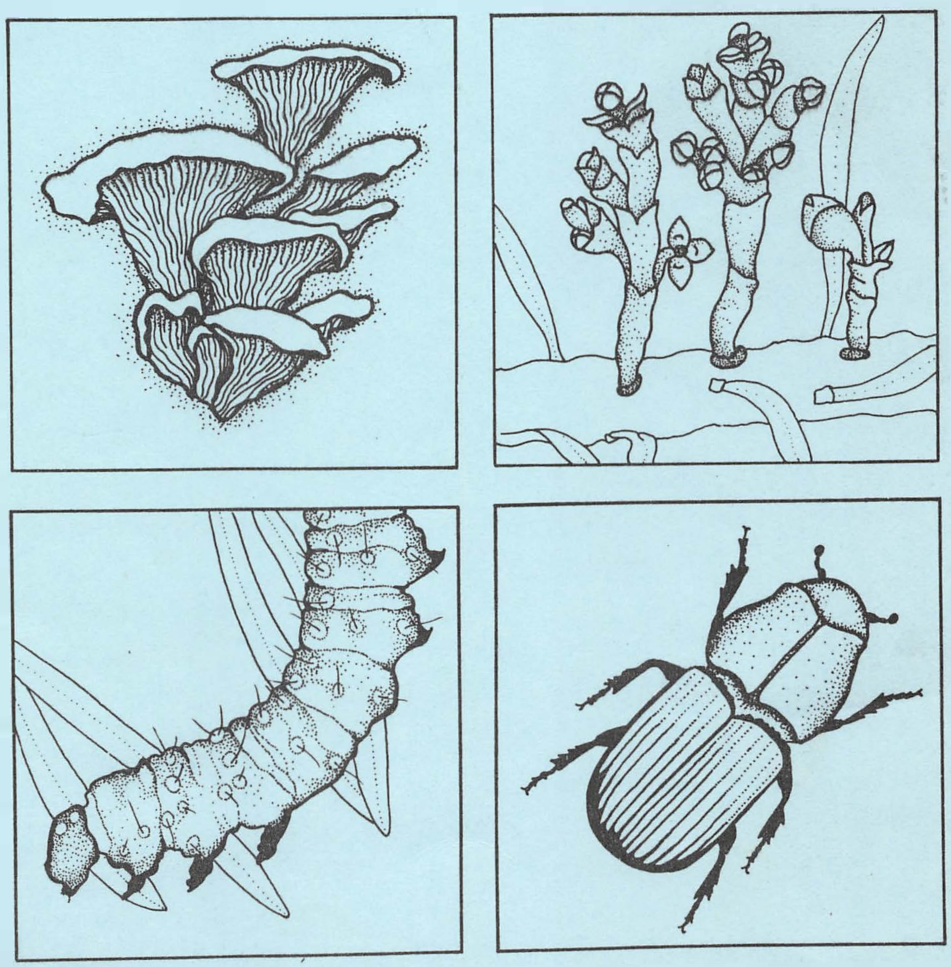

EVALUATION OF FUNGICIDES TO CONTROL BOTRYTIS BLIGHT OF CONTAINERIZED WESTERN LARCH AND LODGEPOLE PINE AT THE COEUR D'ALENE NURSERY, IDAHO

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ABSTRACT

Selected fungicides were evaluated to control *Botrytis* blight of containerized western larch and lodgepole pine seedlings at the Coeur d'Alene Nursery, Idaho. Fungicides were applied at biweekly intervals before and after seedling inoculation with *Botrytis*. Chipco 26019®, Daconil 2787® and Bravo 500® provided the best level of disease control. Tersan 1991® was ineffective. Several fungicides, especially Bravo 500®, restricted height growth of tested seedlings. These tests indicated that several fungicides are available to effectively control this disease when used in conjunction with proper sanitation and cultural procedures.

INTRODUCTION

Botrytis cinerea (Fr.) Pers. commonly causes foliage and twig blight of conifer seedlings in nurseries. The disease is especially severe on containerized seedlings in greenhouses where conditions are ideal for infection and buildup of the fungus (McCain 1978; Thies, et al. 1980). *Botrytis* is usually saprophytic on necrotic tissues (Hancock and Lorbeer 1963; Manning, et al. 1972; Manning et al. 1969). However, the fungus may attack healthy tissues and kill seedlings under humid greenhouse conditions resulting from a thick canopy of closing seedling crowns and overhead irrigation (McCain and Smith 1978).

The disease is common on several coniferous species at the Coeur d'Alene Nursery in northern Idaho. Damage to containerized western larch (*Larix occidentalis* Nutt.) and lodgepole pine (*Pinus contorta* Dougl.) is especially serious. Winter production of larch has been difficult because of *B. cinerea*. Ponderosa pine (*Pinus ponderosa* Laws.) and Engelmann spruce (*Picea engelmanni* Parry.) are also affected. Losses have also occurred at the nursery on larch and spruce within bare root beds, especially in the early cool, wet spring (James 1980).

Botrytis blight has traditionally been controlled in greenhouses by application of fungicides through overhead irrigation (Gillman and James 1980; McCain 1978; McCain and Smith 1978). However, use of high rates and frequent applications have often resulted in tolerance by strains of the fungus to certain of those chemicals (Cooley 1981; Gillman and James 1980; Miller and Fletcher 1974; Dekker 1976; Ogawa et al. 1976). Tolerance to the benzimidazole fungicides has been especially common (Jarvis and Hargreaves 1973; Watson and Koons 1973).

This study was designed to test selected fungicides under greenhouse conditions for control of Botrytis blight; results were to provide a basis for future fungicide registrations. Since the test was initiated, several of the selected fungicides have been registered for use to control *Botrytis* on conifers in Idaho nurseries.

MATERIALS AND METHODS

Six fungicides and a distilled water check were tested against Botrytis blight (table 1). Among these were two formulations of chlorothalonil. All chemicals except iprodione are commonly used on containerzied conifers for *Botrytis* control. Iprodione is a recently marketed chemical, initially designed for turf diseases but with proven effectiveness against *Botrytis* on several greenhouse crops including conifers (A. H. McCain, personal communication).

The chemicals were tested on western larch and lodgepole pine seedlings. Seed were sown in small Leach containers with standard peat-vermiculite growth media during March 1981. Seedlings were thinned after emergence to one seedling per container.

Each fungicide or distilled water was applied to five replicates of 200 seedlings each (standard Leach container tray) for a total of 14,000 seedlings (7,000 for each species) in the test. Fungicides were applied at recommended label concentration (table 1) with a Hudson garden sprayer (20-30 psi) until the solution began to run off the foliage. After treatment, trays were randomly replaced within greenhouses adjacent to nontest seedlings.

Seedlings were treated with fungicides eight times at biweekly intervals beginning May 18. To ensure uniform exposure to *Botrytis* inoculum, all test seedlings were inoculated with a conidial suspension of the fungus on June 4, after two fungicide treatments, when seedlings were about 2 1/2 months old. Spore concentration was approximately 1.15×10^6 conidia/ml in sterile distilled water. Each container tray of 200 seedlings was inoculated with 10 ml of the spore suspension using a fine mist atomizer.

The *B. cinerea* isolate used for these inoculations (CD-16) was obtained from an infected western larch seedling (Coeur d'Alene Nursery). This and several other isolates from different hosts at the nursery were screened for tolerance to several fungicides by S. J. Cooley (Forest Pest Management, Pacific Northwest Region, Portland, OR). These *in vitro* tests involved fungal growth on potato dextrose agar amended with 50 ppm active ingredient of the test fungicide. Tolerance was indicated when the fungus readily grew on the amended medium.

The test was concluded on August 27 when seedlings were at the approximate age and size for shipment to the field. Each seedling was evaluated for survival, infection (based on presence of sporulating *B. cinerea* on foliage), and height to the terminal bud. Treatment effects were compared with an analysis of variance; differences among treatments were located with Duncan's Multiple Range Comparison Test.

Table 1.--Fungicides tested to control <u>Botrytis cinerea</u> on containerized western larch and lodgepole pine at the Coeur d'Alene Nursery.

Trade name	Common name	Chemical name	Application rate per 100 gal. water	Manufacturer
Tersan 1991®	benomyl	Methyl-1-(butylcarbamoyl)-benzimidazole carbamate	1 lb.	Dupont
Botran® 75-W	dicloran	2,6-Dichloro-4-nitroaniline	1 1/3 lb.	Tuco
Bravo 500®	chloro-thalonil	Tetrachlorosiophthalonitrile	2 3/4 pt.	Diamond Shamrock
Daconil 2787®	chloro-thalonil	Tetrachloroisophthalonitrile	1 1/2 lb.	Diamond Shamrock
Captan	captan	N-(Trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide	2 lb.	Stauffer
Chipco 26019®	iprodione	3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboximide	1 lb.	Rhone-Poulene

RESULTS

Relative levels of disease control, represented by percent infection and seedling survival, offered by the tested fungicides, are summarized in tables 2 and 3. Infection indicates any level of *Botrytis* colonization of tissues; survival measures infection severity since seedling mortality was usually due to extensive colonization by the fungus.

Table 2.--Effects of selected fungicides on infection of containerized western larch and lodgepole pine seedlings by *Botrytis cinerea* at the Coeur d'Alene Nursery.

	Western larch		Lodgepole pine	
Fungicide	Seedling infection (percent) 1/	95% confidence interval	Seedling infection (percent) 1/	95% confidence interval
Water	96.2 A	95.0 - 97.4	27.6 A	24.8 - 30.4
Botran®	58.5 B	55.5 - 61.6	0.5 C	0.1 - 0.9
Tersan 1991®	54.8 C	51.7 - 57.9	12.8 B	10.8 - 14.9
Captan	29.7 D	26.9 - 32.6	0.1 C	0.0 - 0.3
Daconil 2787®	8.4 E	6.7 - 10.2	1.7 C	0.9 - 2.5
Chipco 26019®	6.8 E	5.3 - 8.4	0.2 C	0.1 - 0.5
Bravo®	5.9 E	4.4 - 7.4	0.2 C	0.1 - 0.5

1/ Within each column, means followed by the same capital letter are not significantly different (P = 0.05) using Duncan's Multiple Range Comparison Test.

Table 3.--Effects of selected fungicides on survival of containerized western larch and lodgepole pine seedlings at the Coeur d'Alene Nursery.

	Western larch		Lodgepole pine	
Fungicide	Seedling survival (percent) 1/	95% confidence interval	Seedling survival (percent) 1/	95% confidence interval
Captan	97.6 A	96.6 - 98.5	100.0 A	100.0 - 100.0
Chipco 26019®	96.8 AB	95.7 - 97.9	100.0 A	100.0 - 100.0
Daconil 2787®	95.8 ABC	94.5 - 97.0	100.0 A	100.0 - 100.0
Bravo 500®	95.1 BC	93.7 - 96.4	100.0 A	100.0 - 100.0
Botran®	94.0 C	92.6 - 95.5	99.9 A	99.7 - 100.0
Tersan 1991®	87.1 D	85.0 - 89.2	99.6 B	99.2 - 99.9
Water	86.9 D	84.8 - 89.0	100.0 A	100.0 - 100.0

1/ Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Duncan's Multiple Range Comparison Test.

Chipco 26019®, Daconil 2787®, and Bravo 500® provided the best level of disease control in both evaluation categories (tables 2 and 3). Chipco 26019® was especially impressive in controlling the disease (figures 1 and 2). Although this new product has been tested on conifer seedlings only a few times, its performance against Botrytis has been excellent. Both formulations of chlorothalonil (Daconil 2787® and Bravo 500®) were about equally effective in controlling the disease.

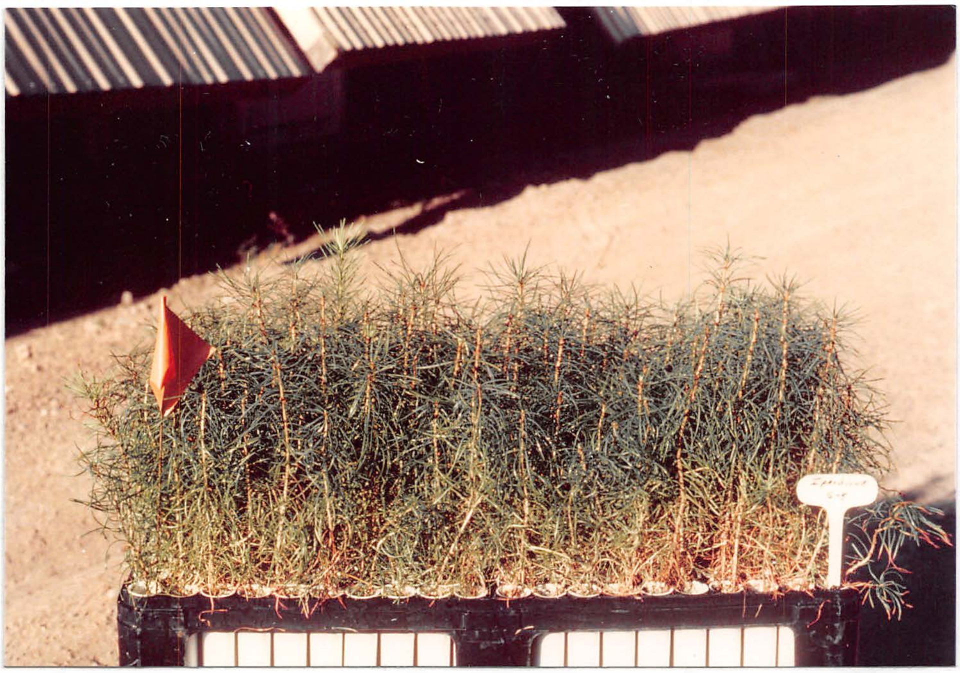

Figure 1.--Control of Botrytis blight of containerized western larch seedlings by Chipco 26019® (iprodione). Note relative absence of seedling mortality and foliar necrosis. Compare with figure 2.

Figure 2.--Botrytis blight of containerized western larch seedlings. Note severe disease levels and extensive foliar necrosis near the base of seedlings in this distilled water (check) treatment.

Although relatively high levels of infection occurred after treatments with captan and Botran® (table 2), associated seedling survival was high (table 3). This probably indicates numerous relatively mild infections which the fungicides kept from becoming severe.

Tersan 1991® did not effectively control Botrytis blight in our tests. Extensive infection resulted (table 2) and the high seedling mortality was similar to the distilled water (check) treatments. All Coeur d'Alene Nursery isolates screened for fungicide tolerance by the Pacific Northwest Region were tolerant to the benomyl formulations tested (table 4). Low levels of tolerance to captan and Daconil 2787® were also indicated. No tolerance to Botran® was found.

Infection and survival levels of western larch and lodgepole pine differed. Larch was much more susceptible. Infections on lodgepole pine were localized with little accompanying fungal growth and tissue necrosis; pine seedling mortality was rare (table 3).

Several fungicides significantly decreased height growth of treated seedlings (table 5). Bravo® caused the greatest impact on height for both western larch and lodgepole pine. This fungicide also caused occasional chlorosis at the tops of seedlings and left heavy residues on the foliage.

Table 4.--Effect of fungicides on radial growth of selected *Botrytis cinerea* isolates from the Coeur d'Alene Nursery. 1/

Fungicide 6/	Manufacturer	*Botrytis cinerea* isolates									
		CD-36 2/		CD-25 3/		CD-16 3/		CD-6 4/		CD-29 5/	
		Growth 7/	Percent 8/	Growth 7/	Percent 8/	Growth 7/	Percent 8/	Growth 7/	Percent 8/	Growth 7/	Percent 8/
Check	-	6.80	100.0	7.23	100.0	7.25	100.0	5.30	100.0	7.30	100.0
Benlate®	Dupont	5.13	75.4	5.33	73.7	5.73	79.0	4.00	75.5	4.22	57.8
Benomyl®	Lilly Miller	5.20	76.5	5.25	72.7	5.75	79.3	3.80	71.7	4.24	58.1
Botran®	Tuco	0	0	0	0	0	0	0	0	0	0
Captan®	Lilly Miller	1.00	14.7	2.25	31.1	1.35	18.6	2.00	37.7	0.24	3.3
Daconil 2787®	Diamond Shamrock	1.05	15.4	3.15	43.6	1.60	22.1	1.85	34.9	2.86	39.2
Ronilan®	BASF	0	0	0.50	6.9	0	0	0	0	0.20	2.7

1/ Data courtesy of S. J. Cooley (Forest Pest Management, Pacific Northwest Region).

2/ Isolated from Douglas-fir.

3/ Isolated from western larch.

4/ Isolated from lodgepole pine.

5/ Isolated from Engelmann spruce.

6/ Fungicides incorporated into potato dextrose agar at 50 ppm active ingredient.

7/ Maximum radial growth, measured in centimeters, of fungus on media after 6 days at room temperature (24° C). Average of 4 replications.

8/ Growth expressed as percent of check.

Table 5.--Effects of selected fungicides on height of containerized western larch and lodgepole pine seedlings.

Fungicide	Western larch		Lodgepole pine	
	Avg. tree hgt. (mm)1/	95% confidence interval	Avg. tree hgt. (mm)1/	95% confidence interval
Tersan®	166.6 A	164.0 - 169.2	119.1 BC	117.6 - 120.7
Water	165.4 A	162.9 - 167.9	125.6 A	124.0 - 127.2
Chipco®	163.8 AB	160.9 - 166.6	120.9 BC	119.2 - 122.6
Daconil®	160.8 BC	158.4 - 163.3	118.6 CD	117.0 - 120.1
Botran®	159.7 C	157.2 - 162.2	121.4 B	119.8 - 123.0
Captan®	153.3 D	150.9 - 155.7	121.3 B	119.7 - 123.0
Bravo®	152.3 D	149.8 - 154.8	116.7 D	115.0 - 118.3

1/ Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Duncan's Multiple Range Comparison Test.

DISCUSSION

The variation of *Botrytis* control obtained from the different fungicides tested indicates that the pathogen apparently developed genetic resistance to some of these chemicals. Substantiating evidence is indicated by the fungicide tolerance screenings done by the Pacific Northwest Region. This is not surprising since other reports (Cooley 1981; Gillman and James 1980) indicated that *B. cinerea* was often very tolerant to several commonly used chemicals.

Although performance trends of tested chemicals were similar on both western larch and lodgepole pine, the low levels of pine infection were disappointing. Using an isolate from larch as the inoculum may partially explain the resulting low pine infection. Also, larch is often damaged more severely by the disease than lodgepole pine and other conifer species.

Tersan 1991® (benomyl), one of the most widely used nursery fungicides against a wide range of diseases (Smith, et al. 1973), did not effectively control *Botrytis* blight in our tests. Tests by the Pacific

Northwest Region showed that tolerance to benomyl was extensive in representative isolates of B. cinerea from the Coeur d'Alene Nursery. Several other reports (Cooley 1981; Gillman and James 1980; McCain 1978; McCain and Smith 1978) indicated that benomyl is no longer effective in controlling Botrytis blight on conifer seedlings, primarily because of acquired resistance to the chemical. In screening for fungicide tolerance, some Botrytis isolates are often relatively susceptible to benomyl, whereas others are quite tolerant (Cooley 1981; Gillman and James 1980). Low levels of tolerance probably exist in a standard population of the fungus and tolerance becomes dominant when selection pressure is exerted by common use of a particular chemical (Webster et al. 1970). Based on the results of these efficacy tests, we cannot recommend continued use of benomyl to control Botrytis blight at the Coeur d'Alene Nursery.

Botran® was not as effective against Botrytis blight as some of the other fungicides, but it satisfactorily controlled the disease. Although tolerance to Botran® was not found in the Coeur d'Alene Nursery isolates screened, previous reports (Cooley 1981; Webster et al. 1970), indicated that B. cinerea can develop tolerance to the chemical. Satisfactory performance from Botran® is probably dependent on rotating or mixing it with other chemicals and levels of Botrytis inoculum available for infection. The chemical provided sufficient disease control to warrant registration for use in the nursery.

Both chlorothalonil formulations (Daconil 2787® and Bravo 500®) provided effective disease control. However, Bravo 500® apparently caused low level phytotoxicity as evidenced by slight chlorosis of seedling tips and reduced terminal growth. Effective disease control was obtained despite common tolerance to chlorothalonil exhibited by several strains of the fungus. Other reports (Cooley 1981; Gillman and James 1980) also indicate common tolerance of Botrytis to the chemical. Therefore, increased tolerance to the chemical should be avoided by using chlorothalonil in combination with other effective chemicals.

Captan is a widely used, general purpose fungicide, effective against many pathogenic fungi (Ogawa et al. 1976). The chemical provided satisfactory control of Botrytis blight in our tests.

Although treated larch seedlings were often infected, survival was high, indicating low disease severity. Low levels of infection can probably be tolerated on seedlings shipped to the field, since damage is likely to decline after trees are outplanted and conditions for intensification by the pathogen are reduced. Therefore, captan should be considered as an effective fungicide against Botrytis.

One of the most satisfactory chemicals tested against Botrytis blight was Chipco 26019® (iprodione) which very effectively controlled the disease. Screening B. cinerea isolates for tolerance to Chipco 26019® was not done; further tests of this chemical are warranted. Another promising new chemical for Botrytis control is Ronilan® (vinclozolin); fungicide tolerance tests indicate that most pathogenic strains are susceptible to the chemical (table 4; Cooley 1981).

These fungicide efficacy tests provide useful information on expected performance of several chemicals against Botrytis blight at the Coeur d'Alene Nursery. Growers should use this information to establish fungicide application schedules that will provide adequate disease control in a cost-effective manner. Alternating the most effective registered chemicals (captan, Daconil 2787®, and Bravo 500®) should help keep disease losses low. Further tests are needed to more completely determine efficacy of Chipco 26019®.

Several cultural practices are necessary to help keep damage from Botrytis at low levels. Reduced stocking density, improved air circulation among plants, and reduced irrigation frequency help create an environment less favorable for the pathogen. Sanitation measures, such as periodic removal of infected plants, will help reduce inoculum levels. These practices coupled with rotating fungicide schedules should help keep losses from Botrytis blight within tolerable limits.

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